

described in the literature. These observations demonstrate that the optimised regenerated silk fibroin prepared according to the protocol of the invention closely resembles native silk fibroin taken directly from the silk gland in the animal and is superior to that prepared by the conventional process described in the literature. It will be appreciated that the ability to form a mesophase is important for allowing the fibroin molecules to be readily orientated in the pore walls during the freezing step.

Example 10

Protocol for Testing the Regenerated Silk Fibroin Solution: Rheological Testing

[0250] Rheometry was used to investigate whether samples of the optimised regenerated silk fibroin solution had rheological properties close to that of native silk fibroin and very different from that of regenerated silk fibroin prepared by the standard protocol disclosed in the literature. The protocol for investigating the rheology of silk fibroin solutions is described below.

Method:—

[0251] A Bohlin Gemini 200 HR Nano rheometer (torque range 10 nNm to 200 mNm, controlled stress/rate viscometry, 3 nNm to 200 mNm, controlled stress/strain oscillation, Malvern Instruments, UK) was used with a cone and plate CP 1/10 (D=10 mm 1° incline). An environmental cuff with moistened tissue was used to prevent the sample drying out. A temperature control unit (Bohlin KTB 30, Malvern Instruments, UK) was used to maintain the temperature at 25° C. Samples were loaded onto the lower plate of the rheometers taking care not to strain the viscous solutions. Optimised regenerated silk fibroin solution was compared with native silk protein obtained directly from the middle division of the final instar *Bombyx mori* silk gland and regenerated silk solutions prepared using the standard protocol described in the literature (see above). All samples had a concentration of approximately 20±1% w/v as determined by gravimetry. To reach this value regenerated silk solutions in dialysis tubes were concentrated by vacuum evaporation at room temperature.

Results:—

[0252] FIG. 4 shows a comparison of the storage moduli of optimised (OBM) regenerated silk fibroin and native silk protein taken directly from the silk gland and standard regenerated silk. The G' and G'' of the optimised regenerated silk fibroin are very close to those of native silk and very different from those of standard regenerated silk fibroin. These parameters for the optimised (OBM) regenerated silk fibroin solution are approximately three orders of magnitude better than the same parameters for regenerated silk fibroin produced through the standard protocol.

[0253] FIG. 5 shows a comparison of the effect of shear rate on viscosity of optimised (OBM) regenerated silk fibroin and native silk protein taken directly from the silk gland and standard regenerated silk. The behaviour of the optimised regenerated silk fibroin solution (OBM Regenerated silk) is closely similar to that of native silk fibroin solution at the same approximate concentration while the viscosity for a

shear rate of 1/s is approximately 4 orders of magnitude higher than that of regenerated silk fibroin produced through the standard protocol.

Conclusion:—

[0254] FIGS. 4 and 5 show that the rheology of the optimised regenerated fibroin solution was closely similar to that of native silk fibroin at the same concentration (approximately 20% w/v) and markedly different from that of regenerated silk fibroin prepared by the standard protocol. These rheological observations clearly demonstrate the vast superiority of the material compared with that prepared using the standard protocol.

Example 11

Protocol for Testing the Regenerated Silk Fibroin Material: Pyrogenicity and Cytotoxicity

[0255] Whole blood assays were performed to assess the pyrogenic activity of samples of the fibroin material.

[0256] The blood assays tested the reactivity of IL-1 α , TNF α and IL-8.

[0257] Data obtained from profiling IL-1 β indicate low levels of pyrogenicity for most of the tested materials, within EU guideline limits. TNF α and IL-8 measurement gave almost identical results to the IL-1 β measurement.

[0258] In conclusion, the results of the tests showed that the samples have low pyrogenicity and display no cytotoxicity.

[0259] With the method of the present invention, the resultant implantable material is capable of carrying out the mechanical functions of meniscal, intervertebral or articular cartilage from the moment of implantation. The high and open porosity combined with resilience enables the material to draw up mesenchymal cells whether these are seeded into the material ex corpore or released into the synovial cavity or space between two vertebral centra after implantation of the material. The excellent biocompatibility and adhesiveness for cells allows them to adhere, grow and differentiate within the pores of the material. Further the material combines high toughness and resilience with slow and tunable resorbability. This enables the material to survive repeated load cycles in situ while mechanical stimulation from normal movements and/or physiotherapy encourages the cells to form new functional tissue with mechanical properties appropriate to and dictated by the local load regime as will be understood by a person skilled in the art. Finally, the smooth and stiff and tough surface of the material provides a low friction surface when lubricated with synovial fluid.

[0260] Although a few preferred embodiments have been shown and described, it will be appreciated by those skilled in the art that various changes and modifications might be made without departing from the scope of the invention, as defined in the appended claims.

[0261] It is of course to be understood that the invention is not intended to be restricted to the details of the above embodiments which are described by way of example only.

1-37. (canceled)

38. A method of preparing a regenerated fibroin solution, the method comprising the steps of:

- (a) treating the silk or silk with an ionic reagent comprising aqueous solutions of one or more of ammonium hydroxide, ammonium chloride, ammonium bromide, ammonium nitrate, potassium hydroxide, potassium chloride, potassium bromide or potassium nitrate;